

The University of Chicago

STUDIES ON THE PHYSIOLOGY OF RECON-
STITUTION IN "PLANARIA LATA," WITH
A DESCRIPTION OF THE SPECIES

A DISSERTATION

SUBMITTED TO THE FACULTY
OF THE ODGEN GRADUATE SCHOOL OF SCIENCE
IN CANDIDACY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOÖLOGY

BY

PRANIS BALTRAS SIVICKIS

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STUDIES ON THE PHYSIOLOGY OF RECONSTITUTION IN *PLANARIA LATA*, WITH A DESCRIPTION OF THE SPECIES.

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The earlier work on the reconstitution of isolated pieces of planarians into new individuals was very largely concerned with the description of the visible changes, such as the outgrowth of new tissue, its differentiation, the reorganization and redifferentiation of the old parts, the changes in shape, the minimum size of pieces capable of reconstitution, and the relations between the polarity of the new individual and that of the animal from which the piece was taken. Most of the experiments were performed with small numbers of pieces, often from different regions of the body and without any attempt at a physiological standardization of the experimental material. During the past twenty years *Planaria dorocephala* has been extensively used in this laboratory as material for experimental investigation, and in the course of this work various methods have been developed which have made possible some degree of physiological analysis of the process of reconstitution in this species. In this work physiological standardization of material, mass experiments, and control of environmental conditions have played an important part.

The desirability of including other species within the program of investigation has become increasingly evident with the progress of the work on *P. dorocephala*, and at the suggestion of Dr. C. M. Child the analysis of reconstitution in a closely related species was begun. The present paper comprises a part of the results of this investigation.

In this connection I take the opportunity to acknowledge my deep indebtedness to Professor Child for his advice, friendly criticism, and revision of the manuscript. I also wish to express my thanks to Dr. L. H. Hyman for the data on oxygen consumption presented in this paper and for many helpful suggestions.

MATERIAL: DESCRIPTION OF SPECIES.

In many rivers and lakes about Chicago a planarian, commonly identified in the past as *P. maculata*, occurs in large numbers. Even a cursory examination makes it evident that this form differs in various respects from *P. maculata* of the Atlantic slope. Attention has already been called to these differences by Hyman ('20). A comparison of living individuals of this form and *P. maculata* from the region of Woods Hole, Mass., shows the following differences: The pigment pattern of the mid-western form (Fig. 1) is distinctly coarser and more irregular; the individual pigment spots and the unpigmented areas are more clearly visible to the naked eye than in *P. maculata* (Fig. 2). In a mixed stock the two

FIG. 1. *Planaria lata* n. sp.FIG. 2. *Planaria maculata*.

forms are at once distinguishable by these differences in pigmentation. Moreover, the general color effect to the naked eye in the mid-western form is a light grayish brown, mottled or dappled, while in *P. maculata* the brown tint is much deeper and more uniform. In *P. maculata* a light median longitudinal stripe is almost invariably present (Fig. 2), while the mid-western form usually shows an obscure dark median stripe (Fig. 1).

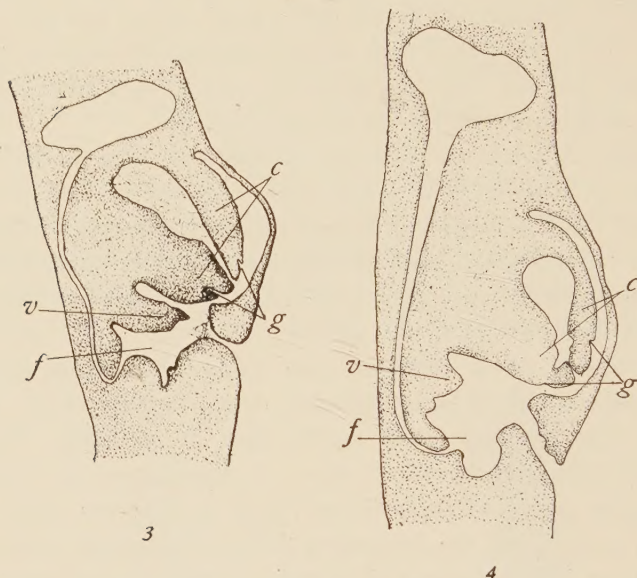
As regards shape of body, the mid-western form is distinctly broader in proportion to length than *P. maculata* (cf. Figs. 1 and 2). This difference is evident during locomotion as well as at rest. The cephalic lobes are apparently slightly less developed and the digestive tract appears more highly branched in the mid-western form than in *P. maculata*.

As regards motor behavior, also marked differences appear. The mid-western form is distinctly more sluggish, reacts more slowly, and progresses less rapidly than *P. maculata*. Similar differences in reaction to food exist. *P. maculata* can be collected by placing pieces of meat in the water in localities where the species occurs. As the extractives diffuse, the animals will come to the meat from a distance of several inches in standing water and from greater distances in flowing water. The mid-western form can not be collected in any considerable numbers in this way because only those animals immediately about the meat react. In feeding stocks in the laboratory it has been found necessary to grind the meat and spread it over the bottom of the container, instead of placing pieces at intervals of two or three inches, as has been the practice with *P. dorotocephala* and *P. maculata*.

All these differences persist unchanged in stocks of the two forms kept in the same water and under the same conditions in Chicago. This is true, not only as regards the original individuals, but also as regards young animals resulting from fission or reconstitution of pieces and animals hatched from eggs. Reconstitution experiments with the two forms also show certain characteristic differences in head frequencies.

These differences are unquestionably sufficient to make it evident that the mid-western form is not *P. maculata*. However, since the morphological characteristics of the organ complex of the genital atrium, and particularly the copulatory organ, are com-

monly regarded as the most trustworthy criterion of specific differences, sections of this region of sexually mature animals have been made.



FIGS. 3, 4. Atrial genital complex of the two species of *Planaria*: Fig. 3, *P. lata*; Fig. 4, *P. maculata* combined from two adjoining sections; *c*, copulatory organ; *f*, female region of atrium; *g*, circular groove or furrow on copulatory organ; *m*, male region of atrium; *v*, valve or fold between the two parts of atrium.

Fig. 3 is a median sagittal section through this region of the mid-western form and Fig. 4 a similar section from *P. maculata*, both from sexually mature animals. The circular groove (*g*) about the apical region of the copulatory organ (*c*) is deeper in *P. lata* and the outline of the fold or valve (*v*) between the common portion of the atrium and the female duct (*f*) is very different in the two species. Other differences in shape of the parts and cavities are evident, but may be due in part to differences in muscular contraction. The differences in the atrial complex confirm the conclusion that the two forms are different species, and since the mid-western form does not agree with descriptions of other species already given, it is evidently an unnamed species and is named and described as follows:

Planaria lata n. sp.—Length of full-grown, sexually mature in-

dividuals averaging 12-14 mm., occasionally 16 mm. Body relatively broad, in full-grown animals width from tip to tip of cephalic lobes and from margin to margin at mouth about one sixth of length. Pigment pattern coarse, irregular, often with obscure dark median stripe, the general effect being mottled or dappled light grayish brown. Cephalic lobes short. Animal sluggish and markedly less sensitive to external factors than either *P. dorotocephala* or *P. maculata*. Structure of organ complex of genital atrium as in Fig. 3.

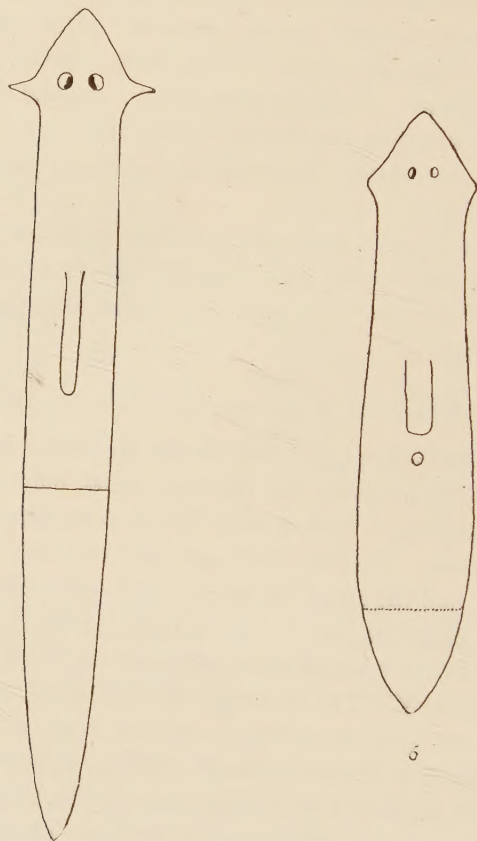
The present paper is primarily concerned with this species, but attention is called to the physiological differences between this species and *P. dorotocephala* which have been brought to light by the experiments.

THE OCCURRENCE OF FISSION AND SEXUAL MATURITY.

Like *P. dorotocephala*, *P. lata* shows no visible morphological indication of the presence of a posterior zoöid, but, as will appear below, the presence of such a zoöid can be demonstrated by physiological methods. Fission in *P. maculata* has been described by Curtis ('02) and the act of fission has been observed in *P. dorotocephala* by Child ('10, '11c). In the latter species it consists in an independent motor reaction of the posterior zoöid while the animal is moving forward. The posterior zoöid attaches itself, the anterior zoöid continues to advance, and the body in front of the attached portion is finally ruptured. Fission is much more likely to occur after slight stimulation than after violent disturbance, for in the latter case the posterior zoöid is controlled by the anterior and does not react independently. The act of fission has not been observed in *P. lata*, but undoubtedly it occurs in the same manner. In the laboratory fission is often induced by changing the water, but does not occur at once, while the animals are very active, but only later as their activity decreases.

As regards the level of the body at which fission occurs, *P. lata* differs markedly from *P. dorotocephala*. In the latter species fission normally occurs at a level 1-3 mm. posterior to the mouth (Fig. 5), and in cases of delayed fission in the laboratory the posterior fission piece may be longer than the anterior. In *P. lata*, however, fission takes place much nearer the posterior end (Fig. 6),

and in animals 12-13 mm. in length the posterior fission piece is only 2-3 mm. in length, and in shorter animals it is not only abso-



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FIG. 5. *P. dorotocephala*, showing level at which fission usually occurs.

FIG. 6. *P. lata*, showing level at which fission usually occurs.

lutely, but relatively, shorter. In *P. dorotocephala* the posterior fission piece often divides again after four or five days, when it begins to move about more or less normally. Apparently at this stage the developing head is unable to control the whole length of the posterior fission piece and fission takes place at one of the more posterior zoöid boundaries. Such second fission has never been

observed in *P. lata*, but the susceptibility (p. 32) and the head frequency (p. 37) of the region just anterior to the level of fission suggest that some slight degree of physiological isolation and the earliest stages of another zoöid exist there in large animals. If such a zoöid is present, it develops to a stage at which fission is possible only after fission has occurred posterior to it and it has become the posterior end of the body.

The maximum length attained by *P. lata* under ordinary conditions in nature and in the laboratory is twelve to fourteen millimeters, and at this size the animals become sexually mature, even though fission continues to occur. The level of fission is so far posterior to the genital pore that the development of the ducts and pore is usually not affected to any appreciable degree by fission. In this respect also this form differs from *P. dorotocephala*, in which the level of fission is so near the level of the genital pore that the occurrence of fission in an animal approaching sexual maturity usually brings about disappearance of the pore and at least the posterior portions of the ducts. In the localities about Chicago *P. lata* becomes sexually mature and deposits eggs from June to September. In the laboratory maturity and egg laying may occur at any time of year. In *P. dorotocephala* sexual maturity may occur in the laboratory when the animals are well fed and fission is prevented by keeping them on slimy or vaselined surfaces, but it has not been observed under natural conditions in this region, being apparently prevented by fission and perhaps also by periodic starvation (Child, '11c). Curtis also found that in some localities *P. maculata* does not become sexually mature, but did not discover the determining conditions. It may be suggested that an environment which inhibits slightly the physiological activity of the animals and so decreases the range of dominance may result in the occurrence of fission at a more anterior level, and this may interfere with the development of the genital ducts and pore. Experiments to test this suggestion have not yet been performed.

METHODS.

The animals are found both on stones at the bottom and on *Elodea* and other water plants at various levels. On the stones all sizes from very young to sexually mature animals and numerous

egg capsules may be found in summer, but among the plants only the smaller, younger animals have been collected. Stocks are kept in the laboratory for three or four weeks before using experimentally so that uniformity in nutritive conditions may be approximated. They are fed three times a week with ground and washed beef liver, as described by Hyman ('20).

The work in this laboratory with planarians has demonstrated that in a stock of animals collected at one time, kept under as nearly as possible identical conditions of temperature, nutrition, water supply, etc., size is the best criterion of physiological condition, and particularly of physiological age, as indicated by susceptibility and respiratory rate, which can readily be applied to the living animals in the selection of material for experiment. Uninjured animals of the same size from such a stock show a high degree of uniformity in susceptibility to chemical and physical agents and in rate of respiration, as shown by the work of Child, Hyman, Behre, and Buchanan, and are more alike physiologically than material selected on any other basis thus far discovered. In these animals the amount of growth, whether rapid or slow, and consequently the size of the individual, is a far more exact measure of their physiological age, and so of their susceptibility and rate of respiration, than the length of time they have lived as individuals (Child, '15a, Chap. IV.; Hyman, '19 C).

Since the experiments recorded in this paper are all mass experiments—*i.e.*, with numbers of individuals—the material for each experiment is selected on the basis of size. Such standardization of material is necessary for the attainment of definite results which can be predicted and controlled and it makes possible prediction and control to a high degree. In the course of the work experiments were performed with standardized material from general stocks collected and kept as described above, from stocks composed of animals hatched from eggs in the laboratory, and from stocks grown from cut pieces.

The experimental data presented below concern chiefly respiration, susceptibility, and head frequencies—*i.e.*, the frequencies of occurrence of the various forms of head in the reconstitution of pieces in relation to level of body, length of piece, and physiological age of animals. Some experiments on modification and control of

head frequency by means of chemical agents have been performed, but are only briefly mentioned.

The data on respiration include comparative estimations of CO_2 production by colorimetric determination of pH with phenol-sulphone-phthalein as indicator and the indicator-buffer solutions made up by Hynson Westcott and Dunning as standards. In these experiments lots of as nearly as possible equal weights of animals or pieces to be compared are sealed in pyrex tubes of the same diameter as the standard tubes in equal volumes of indicator solution of the same concentration as the standard tubes and the change in color recorded at regular intervals and usually also to a definite pH. Some data on oxygen consumption determined by the Winkler method are also given. For these I am indebted to the kindness of Dr. Hyman.

In the experiments on susceptibility KNC has been chiefly used as agent, since it has been abundantly demonstrated that with proper precautions susceptibility to KNC can be used as a general comparative measure of physiological condition and particularly of rate of respiration in *Planaria*.¹ In the present study the susceptibility method has been used for two purposes: first, for demonstration of the axial gradients and the posterior zoöid by the differential susceptibility of different levels of the body; second, to demonstrate the changes in physiological condition of pieces following section. The general susceptibility gradients are shown in figures and the comparative susceptibilities of pieces by graphs. The method of graphing the data is described in connection with the data.

In the experiments on reconstitution the body posterior to the head is cut into a certain number of pieces—four, six, eight, sixteen—according to the experiment. Animals of the same size are used for each experiment and the pieces from each individual are cut as nearly as possible the same length, the more extreme irregularities being discarded. Consequently the corresponding pieces from different individuals represent as nearly as possible the same region of the body. All corresponding pieces—*i.e.*, those

¹ Child, '14a, c, '15a, chaps. III.–VII., '16, '19a, b; Hyman, '19a, b, c, '20, '22.

representing the same body region—are kept together in one container. The usual number of pieces in each lot is fifty, each taken, of course, from a different individual of the size used in that experiment. The pieces are allowed to undergo reconstitution and examination is made and results recorded after twelve to fourteen days, at which time reconstitution is so far advanced that no further change in the character of the form produced will occur. Since the forms produced fall into certain groups or types, as described in a following section, the results can be tabulated to show the frequency of each type in each lot. In this species, as in *P. dorotocephala*, the head is the most characteristic distinguishing feature of the different forms produced, except in very short pieces. The basis of tabulation is, therefore, form and structure of the anterior end, and the tables show the frequencies of the different forms and are called, for convenience, head frequency tables, this term having been used for similar data on *P. dorotocephala*. From the tabulated head frequencies graphs are constructed by a method described below, and in this way the head frequencies in different regions, in pieces of different lengths, and in animals of different size or physiological condition may be directly compared.

The relation of head frequency to region of body and length of piece is shown by comparing pieces of different lengths and from different body levels of animals of the same size, and therefore approximately of the same physiological age. The relation of head frequency to physiological age is determined by comparing the results obtained with pieces from animals of different lengths. In my experiments animals of two standard lengths have been chiefly used: full-grown animals, 11–13 mm. long, physiologically old, and with low rate of respiration; and young growing animals, 4–6 mm. long, with much higher rate of respiration. The smaller size is the smallest which can be conveniently used for such experiments because of the difficulty of cutting pieces of equal size in the smaller individuals. Some experiments have been performed with sizes intermediate between these two extremes, some with animals raised from eggs and some with animals raised from cut pieces.

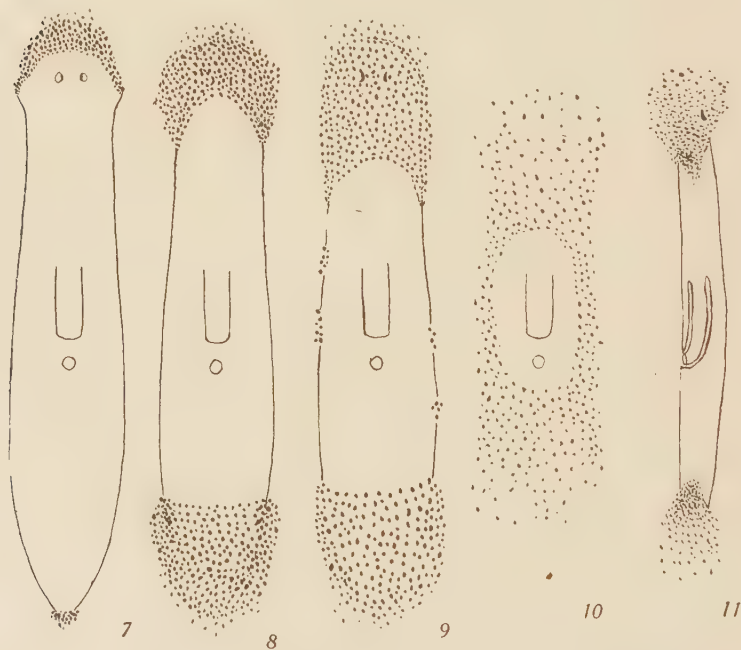
THE AXIAL SUSCEPTIBILITY GRADIENTS.

The occurrence of definite axial susceptibility gradients in both animals and plants and their relation to metabolic rate has been discussed in various publications (*e.g.*, Child, '20b). Various lines of evidence show that susceptibility to a wide range of chemical agents in concentrations or intensities above the limit of tolerance or acclimation and below the limit at which death occurs immediately varies in general directly with metabolic rate, or more specifically with rate of respiration. Susceptibility gradients are characteristic features of physiological axes in both plants and animals and many facts indicate that such axes are primarily quantitative physiological gradients.

It has been found that in *P. dorocephala* the head frequencies at different levels of the body show a definite relation to the polar susceptibility gradient, regions of high susceptibility being regions of high head frequency and vice versa. The observations on susceptibility in *P. lata* show that a similar relation exists in this species. In these observations KNC was used in most cases as agent, because it is known to be a powerful inhibitor of oxidations and has been extensively employed in the study of susceptibility, but many other agents of very different constitution—*e.g.*, HgCl_2 , CuSO_4 , acetic acid, various anesthetics, etc.—in proper concentration give essentially the same results. The procedure consists, first, in determining a concentration which kills slowly enough to permit the differences in susceptibility to appear clearly and which is not low enough to permit acclimation, and, second, in observing the progress of disintegration of the tissues of animals placed in the solution, in closed containers if necessary to avoid loss from volatilization. As death of any part occurs or approaches structural disintegration of the tissues takes place, and such disintegration appears first in certain regions and follows a definite course, so that certain body regions are completely disintegrated while others are still intact and moving.

The general course of disintegration in *P. lata* is shown in Figs. 7–10. The head and the posterior zoöid are most susceptible and disintegration progresses posteriorly from the head and at the same time involves a region anterior to the posterior zoöid and

posterior to the genital pore, the last region to disintegrate being that between the mouth and the genital pore. Commonly the lateral margins of the body disintegrate somewhat earlier than median regions at the same level, but in some individuals these transverse differences do not appear, and in some the median region apparently disintegrates earlier than the margins. There is not much difference between dorsal and ventral, but ventral regions usually disintegrate slightly in advance of dorsal (Fig. 11).



FIGS. 7-11. Susceptibility gradients in *P. lata*, as shown by KNC $m/1000$: Figs. 7-10, stages in progress of disintegration in dorsal view; Fig. 11, a stage of disintegration in lateral view to show difference between dorsal and ventral.

It should be noted that these statements concern primarily the body wall, but they appear to hold good for the parenchyma also. In well-fed animals the digestive tract is highly susceptible and, even though the cyanide must pass through the body wall to reach it, the digestive tract usually swells and disintegrates earlier than the body wall and often bursts through the latter at various points. In starved animals the digestive tract is much less susceptible and

in advanced starvation may remain intact longer than the body wall.

In general, the course of disintegration in this species is very similar to that in *P. dorocephala*, but some minor differences appear. In long individuals of the latter, possessing more than one zoöid in the posterior region, the different zoöids usually show different susceptibilities (Child, '11c, '13b). The early disintegration of the region anterior to the posterior zoöid in *P. lata* may indicate the presence here of another zoöid at a very early stage; in other words, some degree of physiological isolation with increase of metabolic activity may exist anterior to the level of fission and yet be insufficient to permit fission at this level.

In *P. dorocephala* the margins of the body always disintegrate in KNC before median regions, while in *P. lata* this may or may not be the case. These differences are apparently associated with the specialization of the margins as motor organs, and particularly as organs of secretion of slime. There are many glands in this region and the alkaline cyanide stimulates these to increased activity. Often the separate glands disintegrate before other parts of the margins, particularly in *P. dorocephala*. Apparently the margins of *P. lata* are less highly specialized in this way, for the glands appear less distinctly as regions of disintegration and there is less motor activity of the margins. In neutral or acid cyanide and in other acid agents the glandular activity is apparently not increased and motor activity is decreased, and in such solutions the susceptibility of the margins, even in *P. dorocephala*, is usually less than that of median regions.

In *P. dorocephala* the dorsal body wall disintegrates earlier than the ventral in alkaline cyanide. The dorsal wall is thinner than the ventral and shows many localized regions of disintegration, apparently glandular. In acid agents ventral regions are usually more susceptible. In *P. lata* the specialization of the dorsal surface is apparently less, so that even in alkaline agents the difference between dorsal and ventral is slight and the ventral surface is usually the more susceptible.

In the early developmental stages of turbellaria the median ventral region is more susceptible than lateral and dorsal regions (Child, unpublished), and in full-grown planarians the new tissue

develops more rapidly from the median ventral region than from other parts of a cut surface. This fact suggests that internally the median ventral region still possesses the highest metabolic rate. Apparently the primary symmetry gradients undergo more or less alteration in the body wall of some species, and the susceptibility data indicate that such alteration is greater in *P. dorotocephala* than in *P. lata*. The latter species seems to retain more nearly the characteristics of earlier stages.

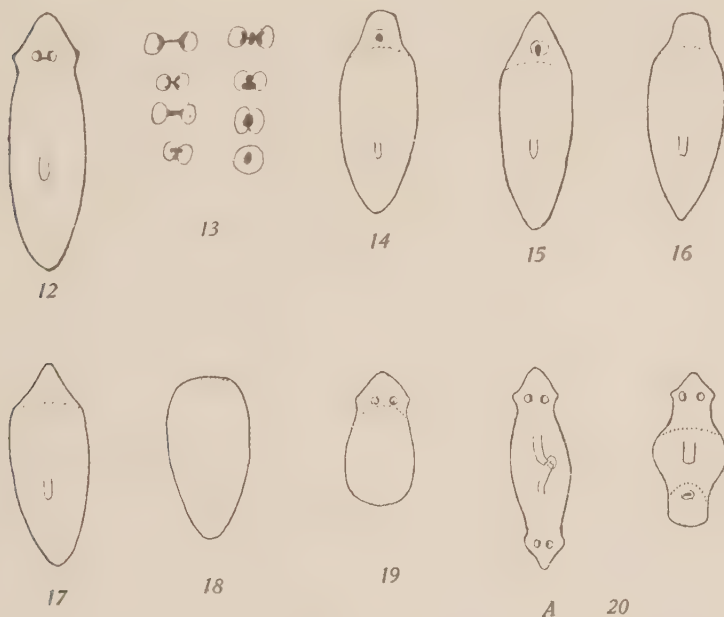
Young animals are always more susceptible than old and the differences in susceptibility at different levels of the body are less in the young. In fed animals susceptibility decreases from the time of hatching to maturity and in this respect parallels rate of respiration (Child, '19a; Hyman, '19c). In full-grown animals the time from the beginning to the end of disintegration in $m/1,000$ KNC at 20° C. is eight to ten hours; in young animals it is much less, increasing with advancing age. High temperature increases, low temperature decreases susceptibility. Starvation increases susceptibility and also decreases the differences at different levels. In all these respects the two species are alike.

THE FORMS RESULTING FROM RECONSTITUTION.

As in *P. dorotocephala*, the results of reconstitution differ in definite and orderly ways according to length of piece and region of body from which it is taken. Some of these differences, such as the level at which the new pharynx appears, length of prepharyngeal and postpharyngeal regions, are merely temporary features of the earlier stages of reconstitution, and are later largely or completely obliterated by differential growth of prepharyngeal and postpharyngeal regions, particularly if the new individuals are fed, until finally all normal or nearly normal individuals attain approximately the same proportions.

The most conspicuous differences in the results of reconstitution concern the head and these differences are, with certain rare exceptions, permanent. Isolated pieces do not always develop anterior ends like that of the normal animal in nature, but abnormal forms occur which constitute a continuous series with some secondary modifications from the normal head to a completely headless con-

dition. The members of this series fall readily into the same types or groups as in the case of *P. dorotocephala* (Child, '11a, '21), viz., normal, teratophthalmic, teratomorphic, anophthalmic, acephalic. The normal head is like the head of Fig. 1. The teratophthalmic head is normal in outline, but the eyes are more or less approximated to the median line and the pigment spots are often partially united or even fused (Figs. 12, 13). In the teratomorphic head there is a single, or apparently single, median eye and the anterior region between the cephalic lobes is incompletely developed, so that the lobes appear more or less anteriorly or fused at the median line (Figs. 14, 15). The anophthalmic head is merely an outgrowth of tissue without eyes (Figs. 16, 17), and in



FIGS. 12-20. Forms resulting from reconstitution: Figs. 12, 13, teratophthalmic heads and eyes; Figs. 14, 15, teratomorphic heads; Figs. 16, 17, anophthalmic heads; Fig. 18, acephalic form; Fig. 19, tailless form; Fig. 20, biaxial heads.

the acephalic form the anterior end simply heals over without outgrowth (Fig. 18). As regards degree of development, the cephalic ganglia of these forms also constitute a continuous series from normal ganglia in the normal head to a rudimentary ganglion in

the anophthalmic, and no ganglia in the acephalic form (Child and McKie, '11). In this species, as in *P. dorocephala*, no other head forms have been observed except secondary modifications of some one of these forms in acclimation to, or recovery from, inhibiting agents (Child, '21) and inequalities or asymmetries in position of eyes resulting from oblique section or other incidental conditions and usually temporary. The frequency of occurrence of these various forms in any lot of pieces is the head frequency of the lot, and the following experiments on reconstitution are chiefly concerned with the relations of head frequency to length of piece, level of body, and size of animal from which pieces are taken.

The results of reconstitution in very short pieces require mention. Forms with heads, usually normal, but without posterior ends, "tailless forms" (Fig. 19) and "biaxial heads" (Fig. 20), appear rarely in sixths of large animals, more frequently in eighths, and their frequency increases with decreasing length of piece to the limit of length at which wound closure and reconstitution fail to occur. Under ordinary conditions these forms are much more frequent and appear in longer fractions of the body in this species than in *P. dorocephala*. In the latter species they have never been seen in sixths or eighths, except rarely in eighths from small young animals, and they are rare even in sixteenths and twentieths of large animals.

HEAD FREQUENCIES IN RELATION TO LENGTH OF PIECE, LEVEL OF BODY, AND PHYSIOLOGICAL AGE OF ANIMAL.

The data presented in this section include head frequencies of fourths, sixths, eighths, and sixteenths of full-grown animals 11-13 mm. in length and fourths and sixths of young animals 4-6 mm. in length. In the series of longer pieces the death rate is negligible, but in the sixths of young and the sixteenths of old animals it becomes high enough to lessen considerably the value of the data on head frequencies, and in pieces shorter than these it is still higher, so that determination of head frequencies becomes impossible in such pieces.

This increasing death rate with decreasing length of piece is certainly to a considerable extent a consequence of increasing area

of cut surface in relation to size of piece. The deaths are practically limited to the first day or two following section. Pieces that survive this period live, with rare exceptions, to the end of the experiment and undergo some degree of reconstitution. In such very short pieces of *P. dorotocephala* the contraction of the cut surface often brings about rupture elsewhere, and contraction at this point produces further rupture in other regions and the piece gradually breaks up. In *P. lata* also the contraction following

TABLE I.
HEAD FREQUENCIES OF FULL-GROWN *P. lata* (11-13 MM.) IN RELATION TO
LENGTH OF PIECE AND LEVEL OF BODY.

Length of Pieces.	Num- ber of Anim- als.	Body Level.	Normal.	Terato- phthal- mic.	Terato- mor- phic.	Ano- phthal- mic.	Aceph- alic.	Dead.	Biaxial Heads.
Fourths.....	210	A	99	—	—	—	1	—	—
		B	47	33	—	6	10	4	—
		C	77	16	—	3	3	1	—
		D	96	1	—	1	1	1	—
Sixths.....	250	A	98	1	—	—	—	1	—
		B	53	30	1	5	9	2	.4
		C	48	33	2	6	8	3	—
		D	43	17	2	8	29	1	—
		E	83	11	1	1	2	2	—
		F	98	1	—	1	—	—	—
Eighths.....	850	A	95	1	—	—	1	3	—
		B	73	16	.5	.5	6	4	.6
		C	46	27	1	3	16	7	.8
		D	37	27	1	8	22	5	.7
		E	39	17	2	6	32	3	1
		F	66	11	2	2	16	3	1.6
		G	85	6	—	1	4	4	.9
		H	96	2	—	—	—	2	—
Sixteenths.....	100	A	72	1	—	—	—	27	—
		B	61	4	—	1	—	34	1
		C	46	2	—	2	4	46	1
		D	47	8	—	2	8	35	3
		E	39	13	—	1	13	34	2
		F	17	13	—	10	22	38	2
		G	22	9	—	7	21	41	1
		H	29	6	—	2	24	39	4
		I	37	5	—	3	26	29	2
		J	26	7	—	9	28	30	3
		K	34	8	—	11	20	27	6
		L	42	8	1	6	10	37	6
		M	45	13	—	5	5	32	6
		N	47	5	—	4	10	34	6
		O	77	5	—	2	7	9	4
		P	88	5	—	—	1	6	—

section is the chief factor in determining these early deaths in short pieces. In the experiments presented here the pieces dying in this manner are included in the totals in calculating percentages and in graphing, but in certain lines of future investigation it will probably be desirable to exclude these early deaths in determining total head frequencies.

The head-frequency data are tabulated in percentages, the full-grown animals in Table I., the young animals in Table II. From

TABLE II.

HEAD FREQUENCIES OF YOUNG *P. lata* (4-6 MM.) IN RELATION TO LENGTH OF PIECE AND LEVEL OF BODY.

Length of Pieces.	Number of Animals.	Body Level.	Normal.	Teratophthalmic.	Teratomorphic.	Anophthalmic.	Acephalic.	Dead.	Biaxial Heads.
Fourths.....	150	A	90	5	—	1	1	3	—
		B	51	17	—	1	15	16	—
		C	72	15	—	—	7	6	.7
		D	85	3	—	1	3	8	—
Sixths.....	150	A	91	3	—	—	1	5	—
		B	61	23	1	1	5	—	—
		C	48	34	—	2	9	7	—
		D	48	23	—	1	17	11	.7
		E	69	6	—	1	5	20	.7
		F	95	4	—	—	1	—	—

the tabulated data graphs are plotted by the method of assigning numerical values to the different head forms as follows: Normal heads, 5; teratophthalmic, 4; teratomorphic, 3; anophthalmic, 2; acephalic, 1; dead, 0. To obtain a head-frequency value for a given lot of pieces, the number of pieces or the percentage of each head form is multiplied by the numerical value of that form and the sum of these products for the particular lot is divided by the number of pieces in the lot, or, if the data are in percentages, by one hundred. The results are the same whether actual number of pieces or percentages are used. These values plotted as ordinates against the successive levels of section, A, B, C, etc., from the heads of the original animals posteriorly as abscissæ, give curves which permit direct comparison of the head frequencies of pieces of different lengths and from different levels.

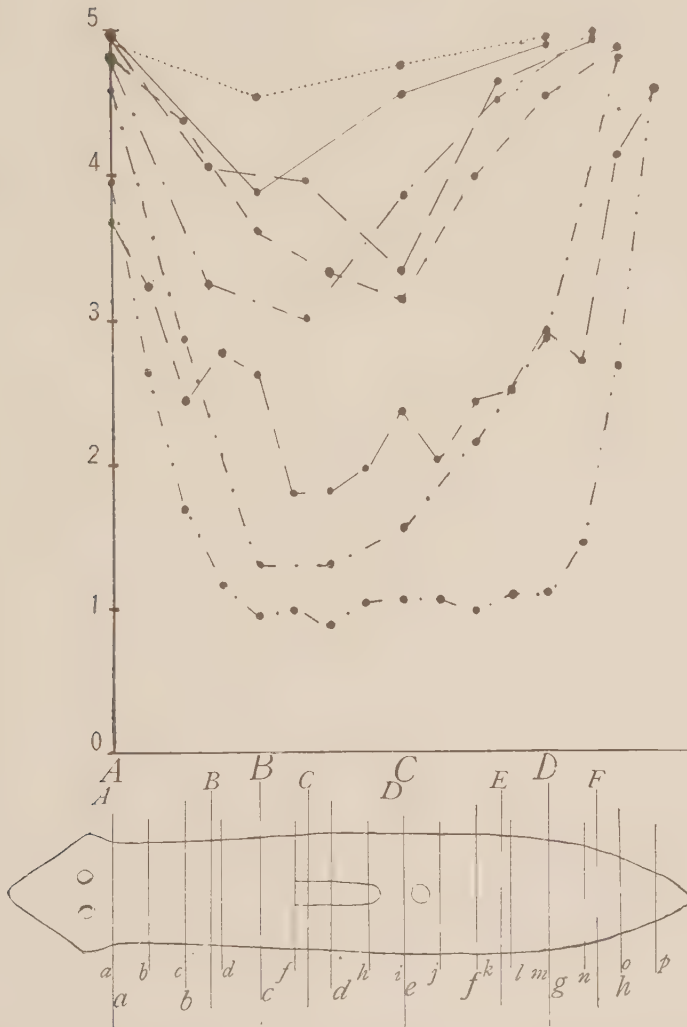


FIG. 21. Head frequencies in relation to length of piece and level of body. *P. lata*: fourths, unbroken line, 110 animals; sixths, long dashes, 250 animals; eighths, short dashes, 850 animals; sixteenths, alternating long and short dashes, 100 animals. *P. dorocephala*: fourths, dotted line, 110 animals; sixths, alternating dots and long dashes, 110 animals; eighths, alternating dots and short dashes, 70 animals; sixteenths, two dots alternating with short dashes, 60 animals. On the outline of *P. lata* below the graph the levels of section of pieces of different length are indicated: A-D, large capitals, fourths; A-F, small capitals, sixths; a-h, large lower case, eighths; a-p, small lower case, sixteenths. The levels of corresponding pieces of *P. dorocephala* are in part slightly different from those indicated in the diagram since in this species the posterior zoöid is much longer and the pharynx therefore nearer the anterior end in large animals than in *P. lata*.

In the graph, Fig. 21, the data for full-grown animals given in Table I. are plotted, together with head-frequency curves from similar experiments with *P. dorocephala*. In Fig. 22 the data for young worms from Table II. are graphed in comparison with corresponding data of *P. dorocephala*.² As regards region of body, the data show, both for full-grown and for young animals, that head frequency decreases from the most anterior level of section posteriorly and then increases again with approach to the level of the posterior zoöid, until at the most posterior level of section it is as high as at the most anterior level, except when lowered by early deaths.

Second, as regards length of piece, the data show for old animals that decrease in head frequency from anterior to posterior region of the first zoöid and increase at levels further posterior becomes greater as length of piece becomes less. The shorter the pieces, the steeper the downward and upward slopes of the curves in Fig. 21. At the most anterior and most posterior levels of the body fourths, sixths, and eighths are practically alike in head frequency, sixteenths somewhat lower anteriorly, but the differences in level of the lowest points of these curves is considerable. The irregularities in the curve of sixteenths are due to the differences in length of the pieces, the variations being, of course, relatively much greater in these very short pieces than in longer pieces. In the young worms (Fig. 22) the differences in steepness in relation to length of piece do not appear in the only data available, those for fourths and sixths, but the sixths show a somewhat lower head frequency than the fourths, except at the posterior end, where it is the same in both.³

² The curves of head frequency of *P. dorocephala* are plotted from data obtained from various sources: fourths, sixths and eighths of old animals and fourths and sixths of young animals from Child ('11b, '16, '20a), one series of eighths of old animals from Miss M. A. Hinrichs and a complete series of my own for both old and young. All of these data are in general agreement as regards relation of head frequency to length of piece, level of body and physiological age.

³ It may be noted that the data for young worms are made up in part from animals raised in the laboratory from eggs and in part from animals of the same size similarly raised from cut pieces. The pieces of animals from eggs showed a somewhat higher death rate and therefore a somewhat lower head frequency than those from cut pieces, but the differences were not great.

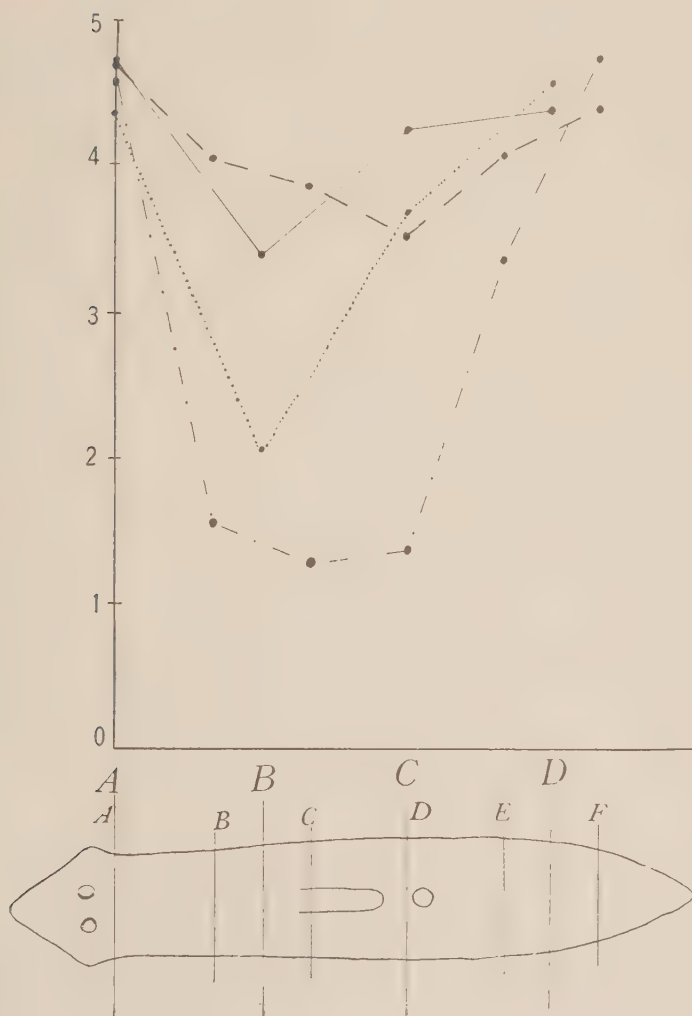


FIG. 22. Head frequencies in relation to length of piece and level of body in young animals. *P. lata*: fourths, unbroken line, 150 animals; sixths, dashes, 150 animals. *P. dorotocephala*: fourths, dotted line, 60 animals; sixths, dots and dashes, 100 animals. The diagrammatic outline below graph indicates levels of section: A-D, large capitals, fourths; A-F, small capitals, sixths.

Since the number of animals obtained from eggs was limited, it was not possible to determine whether these differences were characteristic or merely the result of slight differences in experimental conditions. For the present, therefore, it has seemed best to combine these data in tabulation and graphing.

Comparison of the curves for *P. lata* with those for *P. dorocephala* in Figs. 21 and 22 shows that the changes in head frequency with length of piece and level of body are in general of the same sort, but that their range is very much greater in *P. dorocephala* than in *P. lata*. In full-grown animals (Fig. 21) the head frequencies of fourths of *P. dorocephala* are somewhat higher than, those of sixths about equal to, and those of eighths and sixteenths far lower than the head frequencies of corresponding pieces of *P. lata* from the posterior regions of the first zoöid, while at anterior and posterior ends of the body the differences between the two species are slight. In the young animals (Fig. 22) fourths and sixths from the posterior region of the first zoöid of *P. dorocephala* are far below fourths and sixths of *P. lata* from the same region, while at the anterior end of the body the differences are much less, though greater than in old animals.

The graph, Fig. 23, is a comparison of the curves of fourths and sixths of full-grown and young individuals of *P. lata*. This graph shows that the head frequencies in young animals are slightly lower than in corresponding pieces of old animals, but this age difference is much less than in *P. dorocephala*. Comparison of Figs. 21 and 22 shows that the curves of fourths and sixths of young *P. dorocephala* are much steeper and fall much lower than those of fourths and sixths of old animals.

Tables I. and II. show the frequencies of biaxial heads, but not of tailless forms. Unfortunately tailless forms were not recorded as such in the earlier experiments. It may be stated, however, that they do not appear in the longer pieces, that their frequency increases with decreasing length of piece, and that, so far as data are at hand, they show no definite relation to level of body. As regards the frequencies of biaxial heads, Tables I. and II. show that they do not occur in the longer pieces, that their frequency increases with decreasing length of piece, and is apparently somewhat greater near the level at which fission occurs than elsewhere.

EXPERIMENTAL ALTERATION OF HEAD FREQUENCY.

In *P. dorocephala* the head frequencies have been altered experimentally by many different chemical and physical factors (Behre, '18; Buchanan, '22; Child, '16, '20a). Moreover, it has

been found that head frequencies may be altered in opposite directions in pieces from different levels of the same animals by the

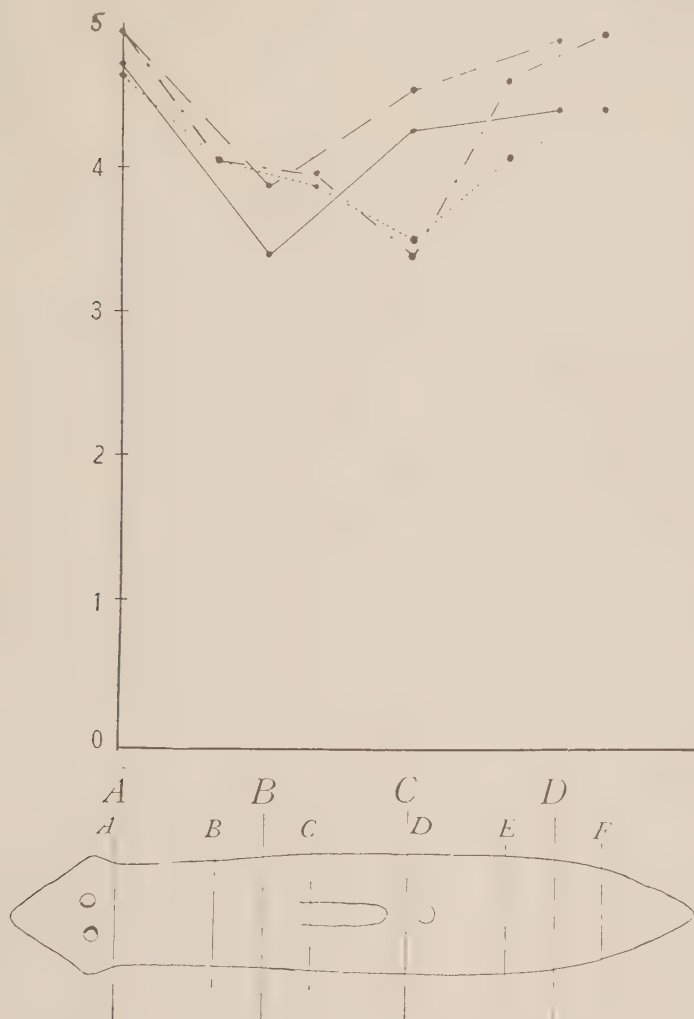


FIG. 23. Head frequencies of fourths and sixths in old and young individuals of *P. lata*: fourths, young, unbroken line; sixths, young, dotted line; fourths, old, long dashes; sixths, old, dots and dashes. The curves in this graph are taken from Figs. 21 and 22.

same concentration of a single agent (Child, '16; Buchanan, '22). In pieces which normally show a high head frequency it may be decreased and in pieces which normally show a low head frequency

it may be increased by the same concentration of cyanide or anesthetic. On the basis of these and many other facts concerning the physiology of reconstitution a theory of head frequency has been advanced (Child, '14a, '14d, '16) which is confirmed by later work (Buchanan, '22). This theory, which is considered more fully below (p. 56), maintains that head frequency in any particular case is determined primarily by the relation between two opposing factors: the one the rate of metabolism in the cells at the cut surface which form the head, the other the rate of metabolism in other parts of the piece. The higher the first in relation to the second, the higher the head frequency; the higher the second in relation to the first, the lower the head frequency. The differential susceptibility of the cells at the cut surface and the other parts of the piece makes it possible through differential inhibition, differential acclimation, and recovery (Child, '20b, '21) to alter the relation between the two factors in both directions.

The correlative factor retarding or inhibiting head formation is apparently in part or wholly a matter of nervous stimulation of the cells not directly concerned in head formation. It is most effective during the first few hours after section, when increased CO_2 production, oxygen consumption and susceptibility all show that the pieces are stimulated. In *P. dorotocephala* this stimulation is inhibited and head frequency increased by anesthetics such as ether and chloroform used during a few hours following section (Buchanan, '22), but a day or two later such anesthetics in the same concentrations bring about no increase (Buchanan, unpublished).

Concerning the experiments on *P. lata*, it may be noted, first, that the two factors are concerned in this species, and, second, that apparently nervous stimulation is less effective in decreasing and nervous inhibition in increasing head frequency than in *P. dorotocephala*. Head frequency can be altered in both directions in *P. lata*, but so far as experiments have gone, it appears that general protoplasmic depressants, such as acids, are highly effective, while anesthetics in the stricter sense have little or no effect. These facts are in accord with observations on the living animal, which indicate that the nervous organization is considerably lower than in *P. dorotocephala*.

Another fact pointing in the same direction is that the original polarity can be more readily obliterated and biaxial heads produced by chemical agents than in *P. dorocephala*. In certain concentrations of acids, for example, the frequency of biaxial heads is much increased.

THE PHYSIOLOGICAL CONDITION OF PIECES FOLLOWING SECTION.

Section of the body results in exposure of a cut surface which gradually contracts and within a few hours cell division and growth begin, giving rise to new embryonic tissue. It has been shown for *P. dorocephala* that increase in rate of respiration and in susceptibility is slight or inappreciable in fourths or longer pieces, while in sixths and shorter pieces it is marked and increases as length of piece decreases, and also increases from anterior to posterior levels of the first zoöid and decreases again in the posterior zoöids (Child, '14a; Robbins and Child, '20; Buchanan, '22; Hyman, '22). Since these changes show definite relations to the polar gradient and are factors in determining head frequency (Child, '14d, '16; Buchanan, '22), it is of interest to determine whether similar changes occur and influence head frequency in *P. lata*.

Changes in Susceptibility Following Section.—The data are most readily presented as graphs, plotted by the method used in earlier work (Child, '15a, p. 81). This method is briefly as follows: Five stages in the progress of disintegration in KNC or some other agent from intact animals or pieces to completely disintegrated are more or less arbitrarily distinguished and these are given, respectively, the numerical values, 40, 30, 20, 10, 0. In determinations of susceptibility a certain number—*e.g.*, ten—of animals or pieces is placed in the solution used and the number or individuals or pieces in each stage is recorded at hourly or half-hourly intervals. The ordinate of the susceptibility curve for any time is the sum of the products obtained by multiplying the number for each stage by the numerical value of that stage, divided by the number of animals or pieces in the lot. These ordinates are plotted against times in hours as abscissæ.

In the experiments on pieces the susceptibility of the fourths, sixths, and eighths was determined separately for each level of the

body; but since the pieces from different levels showed no great differences in susceptibility, the data for all the different levels were brought together in a single curve for each length of piece. Consequently each curve of pieces in the graph, Fig. 24, represents pieces from all levels—i.e., the whole body except the head cut into fourths, sixths, or eighths. KNC $m/1,000$ was used as agent.

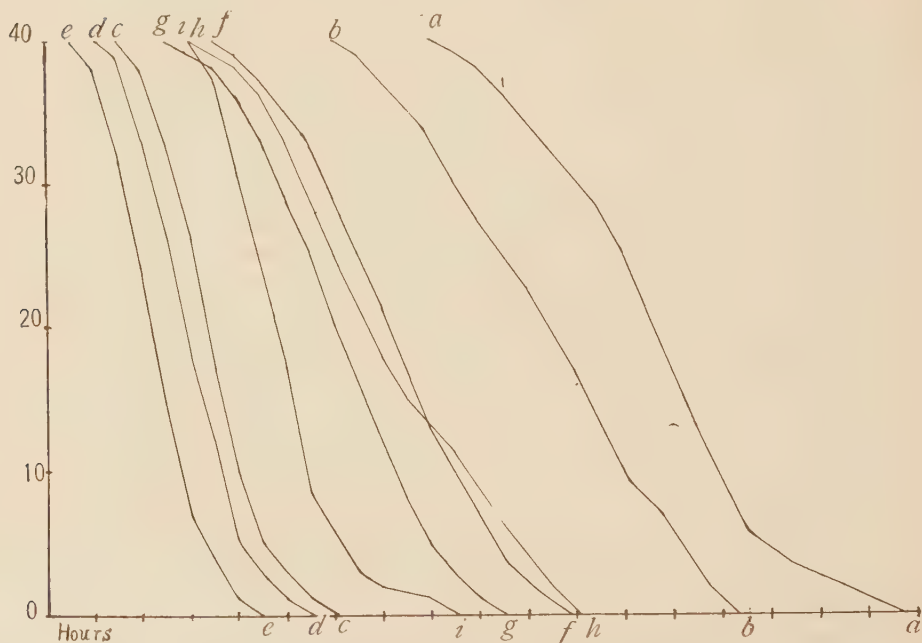


FIG. 24. Graph showing changes in susceptibility following section. Each curve except *aa* and *bb* represents all pieces from 50 animals, *aa*, 50 intact animals, *bb*, 20 headless animals. Further explanation in text.

Fig. 24 shows that susceptibility is greatly increased by section. Uninjured, full-grown animals (*aa*) show the lowest susceptibility of all. Removal of the head increases susceptibility (*bb*). In fourths (*cc*), sixths (*dd*), and eighths (*ee*), immediately after section, susceptibility is greatly increased, and it will be noted that it is highest in eighths, somewhat lower in sixths, and lowest in fourths.

During the first fifteen to twenty hours after section the susceptibility of pieces decreases, then remains nearly stationary for

a day or two, and then gradually rises as reconstitution progresses. Curve *ff* shows the susceptibility of fourths left in water fifteen to eighteen hours after section and then placed in KNC; curve *gg*, that of sixths under the same conditions; curve *hh*, that of eighths after forty hours in water. And, finally, curve *ii* shows the susceptibility of young growing animals 5 mm. long raised from pieces—*i.e.*, approximately the susceptibility attained by fourths after reconstitution is completed—that of sixths and eighths being somewhat higher.

Some part of this increase of susceptibility after section is undoubtedly due to the presence of one (*bb*) or two (*cc*, *dd*, *ee*) cut surfaces. Other experiments for which curves have been plotted, but which are not shown here, demonstrate that pieces with oblique and therefore larger cut surfaces are more susceptible than pieces with transverse surfaces.

The experiments on susceptibility show one other point of importance which does not directly appear in Fig. 24 and which would require a number of graphs for full presentation. It was stated above that pieces of the same length from different levels show approximately the same susceptibility. In the section on susceptibility gradients it was shown that susceptibility decreases from the anterior to the posterior end of the first zoöid and increases again with approach to the level of the posterior zoöid. Even if the susceptibility of pieces from all levels were approximately the same immediately after section, it would be evident that the increase in susceptibility must have been much greater in pieces from posterior than in those from anterior levels of the first zoöid and from the posterior zoöid. It follows from this fact that susceptibility is not simply a matter of the presence of cut surfaces, but depends in part upon level of the body from which pieces are taken.

Comparison of these changes in susceptibility following section with those in *P. dorocephala*⁴ brings to light some interesting physiological differences between the two species. In the first place, removal of the head does not appreciably increase susceptibility in *P. dorocephala*. In fourths it is increased only slightly,

⁴ See Child, '14a. His results have been repeatedly confirmed by myself and by other students in laboratory experiments.

in sixths somewhat more, and in eighths still more, but in all cases much less than in *P. lata*. Moreover, during the first twenty-four hours after section it usually decreases almost or quite to the same level as that of whole animals. In short, the presence of one or even two cut surfaces has in itself little or no effect in increasing susceptibility in *P. dorocephala*. Differences in body level are far more important, particularly in the shorter pieces. Even in sixths or less the increase is slight in anterior pieces, becomes greater toward the posterior end of the first zoöid, and is again slight in the posterior zoöid.

Evidently the increase in susceptibility in relation to cut surfaces and the differential increase at different levels of the body depend, at least in part, on different factors. The former, which seems to be essentially a cellular wound reaction, followed by cell division and growth, is the more conspicuous feature in *P. lata*. The differential increase, on the other hand, is more conspicuous in *P. dorocephala*, but is present also in *P. lata*, and appears to be a stimulation of the piece as a whole. Various facts, such as its short duration and its inhibition by anesthetics (Buchanan, '22), indicate that it is nervous in character. Moreover, it is of interest to note that the differential increase varies inversely as susceptibility at different levels of whole animals and inversely as head frequency at different levels, while the increase in relation to cut surfaces varies directly with area of cut surface in relation to size of piece.

Changes in Rate of CO₂ Production Following Section.—Colorimetric estimations of CO₂ production confirm the data on susceptibility as indicative of changes in rate of respiration. In each of these experiments five 12–13-mm. headless animals entire were compared with five headless animals cut into eighths. The starting point was pH 7.9, and the pH was recorded at regular intervals, and the time required to reach pH 7.3 was also determined. After the experiment both lots were weighed, and in all cases the weight of the pieces was less by some 20 to 50 per cent. than the weight of headless animals, because some loss of intestinal contents, fluids, or even cells occurs when pieces are cut. In all cases, however, the rate of decrease of pH of the pieces was equal to or

higher than that of the entire headless animals. In Table III. the length of time required for change from pH 7.9 to pH 7.3 is given in hours and minutes for ten milligrams of headless animals and pieces, as calculated from the actual weights and times. This method of presentation is open to certain objections, but has the advantage of brevity and clearness. Of the nine experiments in Table III. five were with headless animals and eighths immediately after section, four with headless and eighths twenty-four hours

TABLE III.

TIME IN HOURS AND MINUTES REQUIRED FOR CHANGE FROM pH 7.9 TO 7.3
CALCULATED FOR 10 MG. FROM ACTUAL WEIGHT AND TIME.

Headless.	Eighths. Immediately after Section.	24 Hours a. s.
10:36.....	4:10	—
8:49.....	4:57	—
10:30.....	—	4:10
12:20.....	3:42	—
12:40.....	—	9:20
6:12.....	—	5:12
10:40.....	4:53	—
8:30.....	6:34	—
7:22.....	—	4:44
Mean, 9:48.....	4:51	5:51

after section. In every case the time is much less for the pieces, but is somewhat greater after twenty-four hours than immediately after section. These data confirm the susceptibility data in showing that rate of respiration in eighths immediately after section is greatly increased over that of headless controls, and that after twenty-four hours it is somewhat lower, but still far above that of the controls.

Changes in Oxygen Consumption Following Section.—Table IV. gives the results of determinations of oxygen consumption made by Dr. Hyman. In the first experiment wholes, headless, and pieces are compared, in the others only headless and pieces, the headless animals being used because they show less motor activity than wholes. The pieces include both sixths and eighths from all levels posterior to the head. Table IV. shows that oxygen consumption is greatly increased in pieces immediately after section, as compared with headless animals, and in the first experiment in headless animals as compared with wholes. Twenty-four hours after section the oxygen consumption is lower in some cases, higher

in others, than immediately after section, and increase is more frequent in the pieces than in headless animals. Pieces of *P. dorotocephala* also show a similar increase in oxygen consumption

TABLE IV.

OXYGEN CONSUMPTION OF *P. lata*: WHOLE ANIMALS, HEADLESS, AND SIXTH AND EIGHTH PIECES IMMEDIATELY AND TWENTY-FOUR HOURS AFTER SECTION. CALCULATED IN CUBIC CENTIMETERS OF OXYGEN CONSUMED PER GRAM IN FOUR HOURS.
CALCULATIONS AT 20° C.

Experiment.	Wholes.	Headless.		Pieces.	
		Immediate.	24 Hours.	Immediate.	24 Hours.
1.....	1.31	1.40	1.30	1.93 2.11	1.68 1.78
2.....	—	1.06	.99	1.44	Lost.
3.....	—	1.34	1.47	1.60	1.83
4.....	—	1.26	1.11	1.51	1.71
5.....	—	1.00	1.20	1.59 1.73	1.79 2.18

in some cases (Hyman, '22). In this respect the data on oxygen consumption apparently disagree in part with those on CO₂ production and susceptibility. The reasons for this apparent disagreement are not as yet certainly known, but it seems probable that nutritive condition and growth at the cut surfaces which is appreciable within twenty-four hours are the factors chiefly concerned.

TIME OF HEAD DETERMINATION.

It has been shown that in *P. dorotocephala* the head frequency characteristic of a certain length of piece at a certain level is determined within a few hours after section to such an extent that it is only slightly or not at all altered by decreasing the length of piece after that time (Child, '14d). The experiment to test this point gives essentially the same results in *P. lata*, except that the differences in head frequency in long and short pieces are less than in *P. dorotocephala*. Fig. 25 shows the pieces used in the experiment. A large number of long pieces are cut with anterior ends

at level *aa* (Fig. 25), and at intervals the anterior ends of a certain number of them—*e.g.*, fifty—are cut off as short pieces at the level *bb* and their head frequencies recorded after reconstitution. Omit-

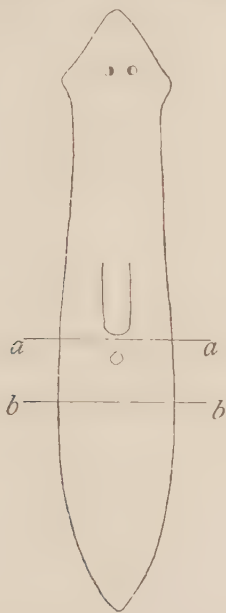


FIG. 25. Outline indicating levels of section, *aa* and *bb*, in experiment on head determination.

ting tables and graphs, the most important results obtained are as follows: In the short pieces isolated at once in the usual manner the chief head frequencies are: normal, 40 per cent.; teratophthalmic, 17 per cent.; acephalic, 30 per cent. In short pieces cut from anterior ends of long pieces after five hours head frequencies are: normal, 80 per cent.; teratophthalmic, 6 per cent.; acephalic, 2 per cent. In short pieces cut in the same way after twenty-three hours 100 per cent. normal heads appeared.

This experiment shows that under ordinary conditions factors determining head frequency are to some extent effective during the first few hours after section, for during this period the alteration of head frequency characteristic of long pieces by great decrease in length of piece becomes progressively less. This, of course, does not mean that head frequency can not be altered in other ways after this time; it merely means that conditions deter-

mining head frequency are to some extent established during this time. Since this is the period of greatest stimulation of pieces, since that stimulation is greater in shorter than in longer pieces and in posterior than in anterior pieces of an individual or zoöid, and since decrease of that stimulation during the first few hours after section increases head frequency, it appears highly probable that the differential stimulation of pieces after section is a factor in determining that under ordinary conditions head frequency is lower in shorter than in longer and in posterior than in anterior pieces of individual or zoöid.

RATE OF GROWTH AND DIFFERENTIATION AND REGIONAL DIFFERENTIALS IN DEVELOPMENT AT ANTERIOR CUT SURFACES.

Only a brief statement of general results along these lines is given at the present time. The growth reaction at anterior cut surfaces is much more rapid in *P. lata* than in *P. dorocephala*. In the former the strong contraction following section has very largely disappeared and a distinct outgrowth of new tissue is present over the whole surface twenty-four hours after section (Fig. 26), while in the latter the cut surface is still strongly contracted and there is less than half as much new tissue (Fig. 27). On posterior surfaces the differences between the two species are less marked (Figs. 26, 27). Two days after section the differ-



26

27

FIGS. 26, 27. New tissue twenty-four hours after section: Fig. 26, *P. lata*; Fig. 27, *P. dorocephala*.

ences between the species remain about the same, but during the third day the rate of growth in *P. dorocephala* becomes more rapid, as compared with that in *P. lata*, and on the fourth day the amounts of new tissue are apparently about the same. Evidently the initiation and acceleration of growth at the cut surface occurs

earlier in *P. lata* than in *P. dorotocephala*. This difference probably accounts, at least in part, for the fact that susceptibility and rate of respiration in pieces of *P. lata* remain considerably higher after section than in whole animals, while in *P. dorotocephala* the decrease within twenty-four hours after section is greater, often to the level of whole animals.

The rate of growth and of differentiation of the head, as determined by the time at which the eyes become visible, is approximately the same in anterior regions and in the posterior zoöid in both species, and in both it is more rapid in these regions than at posterior levels of the first zoöid. It also decreases and the differences in rate at different levels increase with decreasing length of piece. In other words, curves of rate of differentiation of head plotted from repeated observations of developing pieces resemble in their relations to body level and length of piece the head-frequency curves. Comparison of the two species shows, however, that in the shorter pieces and the more posterior levels of the first zoöid the rate of differentiation is slightly lower in *P. lata* than in *P. dorotocephala*. That is to say, in *P. lata* the head frequency in such pieces is higher, but the rate of differentiation of the head is lower than in *P. dorotocephala*.

As regards the portion of body posterior to the head which is formed by new tissue, there is little difference at different levels of section in longer pieces, but with decreasing length of piece this differential appears. In eighths, for example, from levels near the anterior end of the animal and near or in the posterior zoöid, the eyes develop at or slightly anterior to the boundary between new and old tissue (Figs. 28, 29), while in pieces from the poste-



FIGS. 28-30. Regions of body formed by anterior new tissue at different levels in eighths of *P. lata*: Fig. 28, anterior region of first zoöid; Fig. 29, posterior zoöid; Fig. 30, posterior region of first zoöid.

rior region of the first zoöid—*i.e.*, about the level of the pharynx—a considerable portion of the body posterior to the eyes develops from new tissue (Fig. 30). A similar regional differential appears in shorter pieces in *P. dorocephala* and *P. maculata*, but the amount of difference differs somewhat in the different species. In *P. foremanni* and other species which possess no posterior zoöid the regional differential continues to change in the same direction to the posterior end of the body—*i.e.*, the more posterior the piece, the longer the portion of the body formed from new tissue at the anterior end (Morgan, '01).

This regional differential is evidently determined by a complex of factors—*e.g.*, rate of growth of new tissue, size of new head, degree of inhibition in its development by stimulation of the piece, rapidity of reorganization of old parts, and perhaps others. The chief point of interest at present, however, is the fact that this regional differential shows the same relation to body level and length of piece as does head frequency. Its relation to the polar axial gradient is therefore evident. Moreover, experiments show that this differential can be altered and controlled by the same factors by which head frequency is altered and controlled.

DISCUSSION.

Reconstitution in Relation to Body Level, Length of Piece, and Physiological Age.—It is evident that the axial susceptibility gradient is an indicator of fundamental physiological differences along the axis and many facts indicate that such differences are primarily quantitative rather than qualitative. Head frequency, differential increase in susceptibility following section, rate of growth of new tissue at the anterior end, and the portion of the body posterior to the eyes which is formed by new tissue all show a gradation according to body level and therefore a definite relation to the susceptibility gradient. This is true for *P. dorocephala* as well as for *P. lata*. That the physiological factors which determine these graded differences in reaction to section and in the processes of reconstitution are fundamentally quantitative, not qualitative, is indicated by the fact that there is no evidence of fixity or specificity in their relation to body level. All the features of reconstitution characteristic of a given body level under normal

or standard external conditions can be altered to those characteristic of other levels by changes in external conditions which are primarily non-specific and quantitative in physiological effect. This has been shown for *P. dorocephala* in many ways and is also true for *P. lata*, though only a part of the evidence appears in the present paper. It has also been shown for *P. dorocephala* that the susceptibility gradient is an indicator of a corresponding gradient in rate of respiration (Robbins and Child, '20; Hyman, '22), and the data on the changes in susceptibility and rate of respiration in pieces following section leave no doubt that a similar relation between susceptibility and respiratory rate exists in *P. lata*. If this is true, the inference is justified that the reconstitutive differences at different levels are in some way associated with the differences in rate of metabolic reactions, as indicated by rate of respiration.

The relations of reconstitutive processes to length of piece also appear to be non-specific and quantitative in character, for they, too, can be altered by the quantitative action of external factors. And, finally, the relation of head frequency to physiological age is apparently non-specific and quantitative and can be altered experimentally by changes in condition which affect primarily rate, rather than kind of metabolic reactions.

Nowhere do we find any evidence for the existence of specific formative substances. Given the specific protoplasm of a planarian species, the differences in the reconstitutive processes and results are apparently dependent primarily upon quantitative dynamic differences rather than upon specific qualitative factors.

Physiological Analysis of Head Frequency.—It has been shown that the head forms differing from the normal which appear in the reconstitution of *P. dorocephala* represent various degrees of differential inhibition of head development, and that they can all be produced by chemical and physical agents, as well as by physiological factors (Child, '16, '20a, '21; Behre, '18; Buchanan, '22). All the experimental evidence supports the conclusion that two antagonistic factors are concerned in the reconstitution of a head—the one positive or determining, the other negative or inhibitory—and that head frequency in any particular case depends on the relation between these two factors (Child, '14a, '14d, '16, '20a, '21;

Behre, '18; Buchanan, '22). The evidence indicates further that the positive determining factor is the rate of activity of the cells at or near the cut surface (Fig. 31, x) which react to section by



FIG. 31. Diagrammatic outline of piece after section: x , region directly concerned in formation of head; y , region of correlative inhibitory effect on head development.

dedifferentiation, division, and growth, and are directly concerned in head formation. The inhibiting factor, on the other hand, is apparently the correlative influence on x of other parts of the piece (Fig. 31, y) which tends to retard or inhibit the dedifferentiation and growth of the x cells. More or less excitation of the region y occurs temporarily after section, probably largely because of the injury to the nerve cords, and experiments have shown, first, that head frequency decreases as the degree of this excitation increases—*e.g.*, at the more posterior levels of the first zoöid and in shorter pieces—and, second, that inhibition of this excitation increases head frequency. It has been shown further that the differential susceptibility of the regions x and y and the different degrees of excitation of y at different levels of the body provide a physiological basis for altering head frequency experimentally in either direction with the same concentration of a single chemical agent (Child, '16) and with many different agents and conditions (Child, '20a; Behre, '18; Buchanan, '22). In short, the facts indicate that head frequency varies directly with rate of metabolism in x and inversely with rate in y . This relation has been stated in the following brief form:

$$\text{head frequency} = \frac{\text{rate } x}{\text{rate } y}.$$

This formula is perhaps not complete, but serves provisionally to indicate the opposite relation of the two factors to head frequency. If it indicates the relation correctly, it follows that head formation in reconstitution really takes place in spite of the rest of the piece.

In other words, in so far as the x cells become independent of y , they dedifferentiate and begin the development of a new individual, the head arising first as in embryonic development. The various differentially inhibited head forms result from different degrees of inhibition of x by y or by some external factor.

All the experimental evidence at hand indicates that this interpretation holds for *P. lata* as well as for *P. dorotocephala*, the chief difference being that in *P. lata* the inhibiting action of y shows less increase with decrease in length of piece and at more posterior levels of the first zoöid, and that head frequency is therefore normally higher in the shorter pieces and at more posterior levels of the first zoöid and is less increased by inhibition of y in *P. lata* than in *P. dorotocephala*. This difference between the species is what we should expect if the inhibiting action of y on head formation is nervous in character, as the facts lead us to believe. The differences in excito-motor behavior and in development of sense organs certainly indicate a lower degree of nervous organization in *P. lata* than in *P. dorotocephala*. Moreover, the differences in degree of excitation of y following section and in head frequency in relation to level of body and length of piece are less in *P. lata* than in *P. dorotocephala*, and this again suggests a lesser degree of specialization of different body levels in relation to the axial gradient.

The fact that in *P. lata* head frequency is almost as high in young as in old animals, while in *P. dorotocephala* it is much lower in young than in old, also indicates that the region y is less effective in inhibiting head formation at x in *P. lata*. Rate y is higher in relation to rate x in young than in old animals because the tissues of young animals have in general a higher rate of metabolism than those of old, but this difference has much less effect on head frequency in *P. lata* than in *P. dorotocephala*. The more rapid growth reaction in *P. lata* also indicates that y is less effective in this species in inhibiting dedifferentiation and growth of x . The physiological analysis of reconstitution leads, in fact, to the same conclusion as observations on the behavior of the two species, viz., that *P. lata* is a more primitive, a less highly specialized form than *P. dorotocephala*.

Tailless Forms and Biaxial Heads.—Tailless forms develop

from very short pieces in which a single polarity, in these experiments the original polarity, is maintained. They arise when the cells at the posterior cut surface are not sufficiently active in relation to parts anterior to them to grow at the expense of the latter. Heads cut off immediately behind the eyes always remain tailless, and in general when the piece is so short that the posterior cut surface is very close to the new head the development of a new posterior end is inhibited, because the rate of metabolism at other levels of the piece is so high that the cells at the posterior end can obtain but little nutrition.

Biaxial forms arise in very short pieces of *Planaria* when a new physiological gradient arises in relation to the posterior cut surface. In the short piece conditions are most favorable for the origin of such new gradients because there is but little physiological difference between the two cut ends—*i.e.*, these short pieces are nearly apolar because they are short, consequently each cut surface may become a dominant region and determine a polarity in the opposite direction to the other (Child, '15b, pp. 98–100). The higher frequency of biaxial heads under ordinary conditions in *P. lata* than in *P. dorocephala* suggests that polarity—*i.e.*, the longitudinal axial physiological gradient—is less stable in the former, and this also is in accord with the conclusion that *P. lata* is a less specialized form than *P. dorocephala*.

SUMMARY.

1. *Planaria lata* possesses a short posterior zoöid and undergoes fission. Fission does not interfere with sexual maturity, probably because the level of fission is far posterior to the genital pore.

2. Susceptibility decreases from the anterior to the posterior end of the first zoöid and increases again with approach to the posterior zoöid.

3. Head frequency varies in relation to level of body in the same way as susceptibility. The differences in head frequency at different levels increase as length of piece decreases, and head frequency is slightly lower in young than in old animals.

4. Isolation of pieces is followed immediately by a great increase in susceptibility, CO_2 production, and oxygen consumption, then a gradual decrease occurs during 12–24 hours to a level still far

above that of whole animals. Later a gradual increase coincides with the progress of reconstitution.

5. The increase in susceptibility after section in relation to body level and length of piece varies inversely as the susceptibility gradient of whole animals and the head frequency in reconstitution.

6. The factors determining whether a piece shall or shall not develop a head become to some extent effective within a few hours after section.

7. The experimental data all support the conclusion that in *P. lata*, as in *P. dorocephala*, two factors are concerned in determining head frequency: one the rate of metabolism in the cells at the cut surface, being positive or determining; the other the rate of metabolism in other regions of the piece, being negative or inhibitory. Head frequency in any particular case is determined by the relation between these two factors. Apparently the inhibitory factor is less effective in the shorter pieces and at more posterior levels in *P. lata* than in *P. dorocephala*.

8. The physiological analysis of reconstitution agrees with observations on behavior in indicating that *P. lata* is a less highly specialized form than *P. dorocephala*.

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